Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-231-2825.

1. **Grantee Institution:** American College of Radiology

2. **Reporting Period (start and end date of grant award period):** 1/1/2011-12/31/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Stephen M. Marcus, M.S.

4. **Grant Contact Person's Telephone Number:** 267-940-9403

5. **Grant SAP Number:** 4100054841

6. **Project Number and Title of Research Project:** 3 - The Evaluation of Translational Research Program (TRP) Projects

7. **Start and End Date of Research Project:** 1/1/2011-12/31/2014

8. **Name of Principal Investigator for the Research Project:** Kathryn Winter, M.S.

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 171,024.25

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Winter	Principal Investigator	1% Yr 2; 7% Yr 3; 5% Yr 4	$23,838.01
Hunt	Senior Statistician	4% Yr 3	$6,111.30
Moughan	Statistician	7% Yr 3; 20% Yr 4	$36,375.56
Won	Statistician	15% Yr 4	$23,133.52
Zhang	Senior Statistician	4% Yr 4	$7,366.77

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Edgar Ben-Joseph, MD	Investigator	2%
Tim Lautenschlaeger, MD	Investigator	2%
Arnab Chakravarti, MD	Investigator	2%
Charles Kunos, MD	Investigator	2%
Geoff Liu, MD	Investigator	2%
Terrence Williams, MD	Investigator	2%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No___X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No____X_____

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

None

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No_____X_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No___X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not

print as boxes (☐) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

This project aims to use biomarkers and tissue specimens that have been collected in previous RTOG studies to advance current knowledge regarding the treatment and prognosis of cancer patients. The specific research objectives of this project relate to five TRP requests that will contribute to the overall project.

Aim 1: TRP 173: DPC-4 Status in pancreatic cancer patients: RTOG 9704, a Phase III trial of patients with resected pancreatic cancer, is a study that has resulted in several requests from investigators. For this project, the investigators will examine a patient's resected pancreatic cancer with intact DPC-4 to see if there's a local or incompetent metastatic phenotype as well as the correlation of DPC-4 loss with distant tumor recurrence using data collected in RTOG 9704. There will also be an investigation into DPC-4 status that is prognostic for overall survival.

Preliminary analyses were done for this aim. Comparisons of patient and tumor characteristics were conducted using the Chi-squared test. Overall survival was estimated using the Kaplan-Meier method and tested with the log-rank statistic. One-hundred and eight of the 538 eligible patients from the Phase III pancreas trial RTOG 9704 (title) were able to be evaluated for DPC-4. Missing data analyses were conducted and showed no significant differences in patient and tumor characteristics between patients with and without DPC-4 data, although there was a trend towards patients with a primary tumor location of head of the pancreas being associated with DPC-4 scores below the median. Additionally, there was not a significant difference in overall survival for patients with and without DPC-4 data ($HR_{(with/without)}$=1.07; 95% CI: 0.85, 1.36; p=0.57). The distribution of DPC-4 in nuclear AQUA_norm ranged from 773.2 to 5691.4 overall and full distribution statistics by treatment arm and overall are shown in the table below. For this preliminary analysis, a cut point of the median value, a common starting point for a cut point, was used. Based on this cut point, most of the patient and tumor characteristics were balanced between the DPC-4 levels, with the exception of gender. Males were associated with higher levels of DPC-4 (p=0.0021). Two and 5-year overall survival and corresponding 95% CIs for patients with DPC-4 scores below the median were 28% (17%, 40%) and 11% (5%, 21%) respectively; and 43% (29%, 55%) and 25% (14%, 37%) for patients w/ DPC-4 scores above the median, respectively. The log-rank test showed a trend for an association with better overall survival for patients with DPC-4 scores above the median and a clear separation after 1 year, as shown in the figure below.

Distribution of DPC-4 in Nuclear AQUA_Norm

	RT + 5-FU (n=55)		RT + Gemcitabine (n=53)		Total (n=108)	
	n	%	n	%	n	%
DPC-4 in Nuclear AQUA_Norm						
Median	1688.7		2013.2		1753.8	
Min - Max	773.2-5371.4		797.5-5691.4		773.2-5691.4	
Q1 - Q3	1441.1-2847.6		1269.2-3176.2		1273.3-3137.4	
< median (1753.8)	30	54.5	24	45.3	54	50.0
≥ median (1753.8)	25	45.5	29	54.7	54	50.0

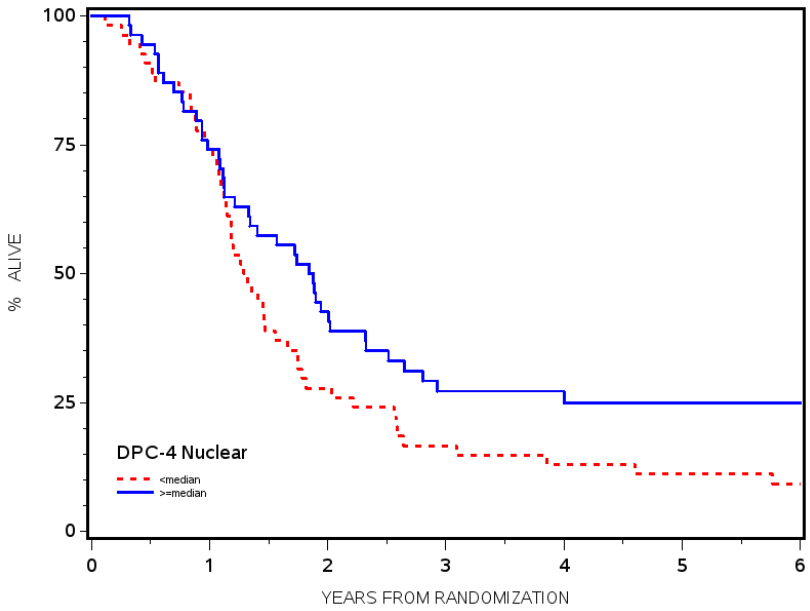
Characteristics of Patients by DPC-4 in Nuclear AQUA_Norm (n=108)

	<median (n=54)	≥ median (n=54)	p-value*
Age (years)			
Median	60	65	
Min - Max	37 - 80.96	35 - 80	
Gender			0.0021
Male	19 (35.2%)	35 (64.8%)	
Female	35 (64.8%)	19 (35.2%)	
Race			0.51
White	48 (88.9%)	50 (92.6%)	
Other	6 (11.1%)	4 (7.4%)	
Primary Location			0.81
Head	44 (81.5%)	43 (79.6%)	
Everything else	10 (18.5%)	11 (20.4%)	

	<median (n=54)	≥ median (n=54)	p-value*
KPS			1.00
80,70,60	20 (37.0%)	20 (37.0%)	
100,90	34 (63.0%)	34 (63.0%)	
T-Stage			0.83
T1,T2	16 (29.6%)	15 (27.8%)	
T3,T4	38 (70.4%)	39 (72.2%)	
N-Stage (surgical)			0.84
N0	18 (33.3%)	19 (35.2%)	
N1	36 (66.7%)	35 (64.8%)	
AJCC Stage			1.00
I,II	18 (33.3%)	18 (33.3%)	
III,IV	36 (66.7%)	36 (66.7%)	
Largest tumor dimension of primary			0.43
< 3 cm	24 (44.4%)	20 (37.0%)	
≥ 3 cm	30 (55.6%)	34 (63.0%)	
Primary tumor status			0.16
Complete resection/negative margins	26 (48.1%)	17 (31.5%)	
Complete resection/positive margins	17 (31.5%)	19 (35.2%)	
Complete resection/unknown margins	11 (20.4%)	18 (33.3%)	
RX			0.34
RT + 5-FU	30 (55.6%)	25 (46.3%)	
RT + Gemcitabine	24 (44.4%)	29 (53.7%)	

*p-value from Chi-square Test

Overall Survival by SMAD4 in Nuclear AQUA_Norm
(n=108)
Log-rank p-value = 0.098



Aim 2: TRP 165: Caveolin-1 and GSK3β in pancreatic cancer patients: Using data and samples collected in RTOG 9704, this project looks to determine whether Caveolin-1, GSK3β and related signaling molecules are prognostic biomarkers with regard to overall survival, disease-free survival, local failure-free survival and distant failure-free survival and correlate Cav-1 expression and pre-operative CA 19-9 levels.

GSK3β is a protein kinase involved in the regulation of cell cycle, transcription, proliferation, differentiation and apoptosis. A number of key oncoproteins, including β-catenin, c-Myc, Cyclin D, Cyclin E, and c-Jun, are known substrates of GSK3β; most are functionally inhibited by it.

Wnt signaling is essential for the embryonic development of the exocrine pancreas and deregulation of this pathway has been linked to pancreatic ductal adenocarcinoma (PDAC). GSK3β is a well-characterized negative regulator of canonical Wnt signaling: it phosphorylates β-catenin, targeting it for degradation. In addition, genomic characterization revealed that 100% of patients with pancreatic cancer have aberrations of the Wnt or Notch pathways.

It has previously been shown that inhibition of GSK3β in a preclinical PDAC model causes stabilization and nuclear translocation of β-catenin, and induces poor differentiation, proliferation, and resistance to radiation. To further explore the potential utility of GSK3β as a prognostic biomarker of clinical outcomes, its cytoplasmic

expression was examined in a tissue microarray (TMA) generated from patients enrolled in Radiation Therapy Oncology Group (RTOG) 97-04, a prospective intergroup multicenter phase III trial of adjuvant chemotherapy and chemoradiation for resected PDAC.

The eligibility criteria for RTOG 97-04 included histologically confirmed PDAC, pathological stages T1–4, N0–1, M0, gross total tumor resection, Karnofsky performance status of ≥60 and adequate hematologic, renal, and hepatic function. After resection, patients were randomly assigned to either continuous infusion 5-fluorouracil (5-FU), 250 mg/m2/day, for 3 weeks (arm 1) or gemcitabine, 1000 mg/m2, 30 minute infusion once weekly for 3 weeks (arm 2) before and after chemoradiotherapy (CRT). CRT was identical in both arms. GSK3β assay was assessed using the HistoRx AQUA® platform. Slides were stained for cytokeratin 8, GSK3β (AbCam, Cambridge, MA, AB31826, clone M131, 1:600), and the DNA staining dye 4', 6-diaminodo-2-phenylindole (DAPI). Images of each core were captured with a microscope at 3 different extinction/emission wavelengths. Within each core, areas of tumor were distinguished from stroma and necrotic areas by creating a tumor-specific mask based on the cytokeratin stain. DAPI image was used to differentiate between cytoplasmic and nuclear staining within the tumor mask. The pixel intensity of the GSK3β protein/antibody complex was then machine-read and reported.

Overall survival (OS) was calculated from date of randomization to date of death due to any cause or last follow-up for censored patients. Disease-free survival (DFS) events were defined as local, regional or distant relapse, appearance of a second primary lesion or death due to any cause. DFS was calculated from date of randomization to date of first documented failure or last follow-up for censored patients. GSK3β was analyzed as a categorical variable using its upper quartile (Q3) as a cut point (<Q3 vs. ≥Q3). This threshold was chosen because it was hypothesized that, as a negative regulator of Wnt, substantial expression would be required for it to exert an effect, an assertion confirmed in an analysis of an independent smaller TMA annotated with clinical outcomes from University of Michigan. The selection of Q3 was based on the results of that exploratory analysis. Potential associations between baseline characteristics and GSK3β groupings were carried out using the chi-square or Fisher's exact test. Univariate and multivariate Cox proportional hazards models were used to determine if there are any associations between GSK3β with OS and DFS. For the multivariate analysis, only GSK3β was forced into the models and a stepwise selection procedure was used to choose other variables using α=0.05 level as the entry and exit criteria for the model building. The following variables were assessed in the models along with GSK3β: treatment arm, age, gender, race, primary tumor location, nodal status, largest tumor dimension, and surgical margin status. All analyses were completed using SAS (version 9.2 for Windows, SAS Institute, Cary, NC) and R (version 2.14 for windows, R Foundation for Statistical Computing).

GSK3β was assayed in a TMA developed from 220 patients. Of these, 57 patients were excluded from the analysis; 21 did not meet eligibility requirements for RTOG 9704 and 36 had failed the AQUA quality test. This left 163 eligible and analyzable patients.

There were no statistically significant differences in baseline characteristics of 163 eligible and GSK3β analyzable cases and all other eligible cases on RTOG 97-04. Similarly, baseline characteristics were not significantly different among patients in the upper quartile (≥Q3 [≥ 2553.64]) and lower three quartiles (<Q3 [<2553.64]) for GSK3β expression. The 3-yr. OS rates (95% CI) for GSK3β <Q3 vs. GSK3β ≥Q3 were 16% (10%-23%) and 30% (17%-44%), respectively [log rank p-value=0.0082 (Figure 2a)]. Patients with GSK3β ≥Q3 have a 41% decrease in the risk of dying than those with GSK3β <Q3 (HR=0.59, 95% CI: [0.40, 0.88], p-value=0.0090). The 3-yr. DFS rates for those with GSK3β <Q3 and GSK3β ≥Q3, were 9% (5%-15%) and 20% (9%-33%) respectively [log-rank p-value =0.0081. Patients with GSK3β ≥Q3 had a 39% decrease in the risk of disease recurrence as compared to patients with GSK3β <Q3 (HR=0.61, 95% CI: [0.42, 0.88], p-value=0.0087). Potential correlations between GSK3β expression and CA19-9 and tumor grade were tested. There were no statistically significant correlations. GSK3β was significantly associated with OS (as were surgical margins, age and CA-19-9). Patients with GSK3β ≥Q3 have 46% reduced risk of dying of pancreatic cancer than patients with GSK3β <Q3 (HR=0.54, 95% CI [0.31-0.96], p-value= 0. 034). No other variables (including treatment arm, nodal status, and tumor diameter) were significantly associated with OS. GSK3β expression had a borderline-significant association with DFS, with a HR of 0.65 (0.98, 1.07; p-value=0.092) while surgical margins and CA-19-9 were statistically significant. To determine if GSK3β is a prognostic factor or a predictor of outcomes in PDAC, the analyses above were also conducted within each treatment arm separately. There were no significant differences in the observed effects by treatment arm.

In summary, these results show that GSK3β is a strong prognostic biomarker in PDAC, independent of other known factors such as T stage, nodal status, surgical margins and CA-19-9. This is the first time a biomarker is clearly implicated as a conveyer of poor prognosis in this disease and represents an important step in the direction of personalized therapy for pancreatic cancer. GSK3β should be considered for stratification in future clinical trials. The findings also raise an intriguing question of whether Wnt signaling should be targeted therapeutically in this disease.

Aim 3: TRP 167: Pharmacogenetic correlative science: The final project using data from RTOG 9704 has an overall goal to identify heritable, germline polymorphic markers that are prognostic and predictive of toxicity in pancreatic cancer patients. Efficacy and toxicity of previously identified putative germline genetic polymorphisms in this patient population will be examined.

Being able to identify heritable, germline polymorphic markers that are prognostic for or predictive of outcome and/or toxicity in resected pancreas patients is of interest. RTOG 9704 is a phase III randomized trial comparing fluorouracil versus gemcitabine before and after chemoradiotherapy as adjuvant therapy for patients with resected pancreatic cancer. The results revealed a non-significant improvement in survival for the gemcitabine arm. Pharmacogenetic studies can identify one or more genetic variations that are highly associated with, and therefore may predict for either drug toxicity or

efficacy. In addition, germline polymorphisms may be prognostic markers of outcome, independent of therapy.

Analyses done so far have focused on the amount of missing data for each polymorphic marker; allele frequencies, including the minor allele frequency; observed genotype numbers; expected genotype frequencies; expected genotype numbers, and an exact test for the Harvey-Weinberg Equilibrium (HWE).

Statistical comparisons to assess potential associations between baseline characteristics and those patients with and without polymorphic marker data were carried out using the chi-square test. The following baseline characteristics were dichotomized: pathological t-stage (T1, T2 vs. T3, T4) and AJCC stage (I, II vs. III, IV). Race was categorized as white vs. African American/other. Univariate analysis of overall (OS) and disease-free survival (DFS) comparing patients with and without polymorphic marker data was also performed. OS and DFS were estimated univariately with the Kaplan-Meier method and polymorphic marker status (with vs. without) were compared using the log-rank test. Cox proportional hazards models were utilized to identify the impact of patients with and without polymorphic marker data on OS and DFS. Given the numerous polymorphic markers and to adjust for multiple comparisons in this analysis, a p-value < 0.001 will be considered statistically significant. A p-value < 0.001 denotes a violation of the HWE.

Patients who are not analyzable are more likely to have head of pancreas tumors, as compared to body/tail of pancreas tumors, than patients who are analyzable (89.0% vs. 81.5%. p=0.024). There were no other statistically significant differences seen in baseline characteristics. There are no statistically significant differences in OS or DFS between those who were analyzable and those who were not analyzable. Sixty-one markers were analyzed, 52 assessed in the lab by the Sequenom assay, 5 by the SNaPshot assay, and 4 by the Sangar assay. Tables were created showing the observed genotype frequencies, the expected genotype frequencies, the calculated allele frequency, and the p-value for testing whether the results violate the HWE and are shown below. Of the 61 markers analyzed, the HWE was only violated for two, both assessed by the SNaPshot assay, and those 2 markers will not be evaluated further.

Genotype method = Sequenom

Genes and SNPs by Sequenom Assay

Marker	RS #	Genotype	Observed Genotype n	Expected Genotype Frequency	Expected Genotype n*	Exact Test for HWE p-value
LOC100131418	rs1021584	CC	101	0.5799	100	1.00
		CT	63	0.3632	63	
		TT	10	0.0569	9	
		Total	174			
		Allele Frequency	C=0.7615			
			T=0.2385	MAF		
		X (failed)	4 (2.2%)			
NR5A2	rs12029406	CC	76	0.4076	72	0.25
		CT	74	0.4617	81	
		TT	27	0.1307	23	
		Total	177			
		Allele Frequency	C=0.6384			
			T=0.3616	MAF		
		X (failed)	1 (0.6%)			
SHH	rs1233556	CC	128	0.7292	129	0.38
		CT	48	0.2495	44	
		TT	2	0.0213	3	
		Total	178			
		Allele Frequency	C=0.8539			
			T=0.1461	MAF		
		X (failed)	0			
TP73	rs1801174	CC	1	0.0103	1	1.00
		CT	34	0.1827	32	
		TT	142	0.8070	142	
		Total	177			
		Allele Frequency	C=0.1017	MAF		
			T=0.8983			
		X (failed)	1 (0.6%)			
MSH2	rs2303428	CC	4	0.0062	1	0.0098
		CT	18	0.1452	23	
		TT	143	0.8486	140	
		Total	165			
		Allele Frequency	C=0.0788	MAF		
			T=0.9212			
		X (failed)	13 (7.3%)			
TREX1	rs3135941	CC	9	0.0275	4	0.03
		CT	41	0.2765	49	
		TT	128	0.696	123	
		Total	178			
		Allele Frequency	C=0.1657	MAF		
			T=0.8343			
		X (failed)	0			

Genes and SNPs by Sequenom Assay (continued						
Marker	**RS #**	**Genotype**	**Observed Genotype n**	**Expected Genotype Frequency**	**Expected Genotype n***	**HWE p-value**
Hent1	rs324148	CC	100	0.5604	99	0.69
		CT	65	0.3764	66	
		TT	12	0.0632	11	
		Total	177			
		Allele Frequency	C=0.7486			
			T=0.2514	MAF		
		X (failed)	1 (0.6%)			
DCTD	rs4742	CC	21	0.1194	21	1.00
		CT	81	0.4523	80	
		TT	76	0.4284	76	
		Total	178			
		Allele Frequency	C=0.3455	MAF		
			T=0.6545			
		X (failed)	0			
NOX4	rs497279	CC	13	0.0747	12	0.69
		CT	62	0.3972	63	
		TT	86	0.5281	85	
		Total	161			
		Allele Frequency	C=0.2733	MAF		
			T=0.7267			
		X (failed)	17 (9.6%)			
TP73	rs5031052	CC	174	0.9775	173	0.017
		CT	2	0.0223	3	
		TT	1	0.0001	0	
		Total	177			
		Allele Frequency	C=0.9887			
			T=0.0113	MAF		
		X (failed)	1 (0.6%)			
MRP5(ABCC5)	rs7636910	CC	16	0.1156	20	0.18
		CT	87	0.4488	78	
		TT	72	0.4356	76	
		Total	175			
		Allele Frequency	C=0.34	MAF		
			T=0.66			
		X (failed)	3 (1.7%)			
IL17F	rs763780	CC	0	0.0011	0	1.00
		CT	12	0.0655	11	
		TT	165	0.9334	165	
		Total	177			
		Allele Frequency	C=0.0339	MAF		
			T=0.9661			
		X (failed)	1 (0.6%)			

Genes and SNPs by Sequenom Assay (continued						
Marker	**RS #**	**Genotype**	**Observed Genotype n**	**Expected Genotype Frequency**	**Expected Genotype n***	**HWE p-value**
Hcnt3	rs7867504	CC	22	0.1136	20	0.61
		CT	76	0.4469	79	
		TT	80	0.4395	78	
		Total	178			
		Allele Frequency	C=0.3371	MAF		
			T=0.6629			
		X (failed)	0			
PYCARD	rs8056505	CC	26	0.1273	21	0.18
		CT	70	0.4589	78	
		TT	75	0.4138	70	
		Total	171			
		Allele Frequency	C=0.3567	MAF		
			T=0.6433			
		X (failed)	7 (3.9%)			
MSH6	rs1800935	CC	12	0.0853	15	0.28
		CT	80	0.4136	73	
		TT	86	0.5011	89	
		Total	178			
		Allele Frequency	C=0.2921	MAF		
			T=0.7079			
		X (failed)	0			
CDA	rs1048977	CC	86	0.4790	84	0.72
		TC	73	0.4262	75	
		TT	18	0.0948	16	
		Total	177			
		Allele Frequency	C=0.6921			
			T=0.3079	MAF		
		X (failed)	1 (0.6%)			
SSTR5	rs169068	CC	54	0.2789	49	0.23
		TC	80	0.4984	88	
		TT	44	0.2227	39	
		Total	178			
		Allele Frequency	C=0.5281			
			T=0.4719	MAF		
		X (failed)	0			
TP73	rs1801173	CC	107	0.6256	110	0.11
		TC	66	0.3307	58	
		TT	4	0.0437	7	
		Total	177			
		Allele Frequency	C=0.791			
			T=0.209	MAF		
		X (failed)	1 (0.6%)			

Genes and SNPs by Sequenom Assay (continued						
Marker	**RS #**	**Genotype**	**Observed Genotype n**	**Expected Genotype Frequency**	**Expected Genotype n***	**HWE p-value**
DCK	rs4694362	CC	27	0.1379	23	0.26
		TC	73	0.4669	79	
		TT	71	0.3952	67	
		Total	171			
		Allele Frequency	C=0.3713	MAF		
			T=0.6287			
		X (failed)	7 (3.9%)			
EXO1	rs735943	CC	44	0.2416	43	0.77
		TC	87	0.4999	88	
		TT	47	0.2585	46	
		Total	178			
		Allele Frequency	C=0.4916	MAF		
			T=0.5084			
		X (failed)	0			
15q14	rs8028529	CC	9	0.0461	7	0.65
		TC	55	0.3372	57	
		TT	106	0.6167	104	
		Total	170			
		Allele Frequency	C=0.2147	MAF		
			T=0.7853			
		X (failed)	8 (4.5%)			
ERHB1	rs36064	CC	27	0.1547	27	1.00
		TC	86	0.4772	84	
		TT	65	0.3681	65	
		Total	178			
		Allele Frequency	C=0.3933	MAF		
			T=0.6067			
		X (failed)	0			
SSTR5	rs4988487	CC	158	0.8908	158	1.00
		TC	20	0.1060	18	
		TT	0	0.0032	0	
		Total	178			
		Allele Frequency	C=0.9438			
			T=0.0562	MAF		
		X (failed)	0			
CACNA2D3	rs11130399	GG	64	0.3792	67	0.43
		GT	90	0.4732	83	
		TT	23	0.1476	26	
		Total	177			
		Allele Frequency	G=0.6158			
			T=0.3842	MAF		
		X (failed)	1 (0.6%)			

Genes and SNPs by Sequenom Assay (continued						
Marker	**RS #**	**Genotype**	**Observed Genotype n**	**Expected Genotype Frequency**	**Expected Genotype n***	**Exact Test for HWE p-value**
RRM1	rs183484	GG	33	0.2094	37	0.29
		GT	96	0.4964	87	
		TT	48	0.2942	52	
		Total	177			
		Allele Frequency	G=0.4576	MAF		
			T=0.5424			
		X (failed)	1 (0.6%)			
IQGAP2	rs3797418	GG	78	0.4645	78	1.00
		GT	73	0.4341	72	
		TT	17	0.1014	17	
		Total	168			
		Allele Frequency	G=0.6815			
			T=0.3185	MAF		
		X (failed)	10 (5.6%)			
TREX1	rss11797	AA	27	0.144	23	0.32
		AG	72	0.471	78	
		GG	67	0.385	63	
		Total	166			
		Allele Frequency	A=0.3795	MAF		
			G=0.6205			
		X (failed)	12 (6.7%)			
MSH3	rs26279	AA	88	0.5137	88	0.85
		AG	72	0.4060	70	
		GG	13	0.0802	13	
		Total	173			
		Allele Frequency	A=0.7168			
			G=0.2832	MAF		
		X (failed)	5 (2.8%)			
MSH3	rs27494	AA	87	0.5072	87	1.00
		AG	71	0.4099	70	
		GG	14	0.0828	14	
		Total	172			
		Allele Frequency	A=0.7122			
			G=0.2878	MAF		
		X (failed)	6 (3.4%)			
MSH2	rs4987188	AA	0	0.0001	0	1.00
		AG	3	0.0186	2	
		GG	157	0.9813	157	
		Total	160			
		Allele Frequency	A=0.0094	MAF		
			G=0.9906			
		X (failed)	18 (10.1%)			

Genes and SNPs by Sequenom Assay (continued)						
Marker	**RS #**	**Genotype**	**Observed Genotype n**	**Expected Genotype Frequency**	**Expected Genotype n***	**HWE p-value**
TGM3	rs6082527	AA	135	0.7485	133	0.33
		AG	38	0.2333	41	
		GG	5	0.0182	3	
		Total	178			
		Allele Frequency	A=0.8652			
			G=0.1348	MAF		
		X (failed)	0			
PARD6G	rs7243052	AA	17	0.1001	17	0.86
		AG	78	0.4326	76	
		GG	82	0.4673	82	
		Total	177			
		Allele Frequency	A=0.3164	MAF		
			G=0.6836			
		X (failed)	1 (0.6%)			
MLH1	rs9876116	AA	52	0.3193	53	0.64
		AG	87	0.4915	83	
		GG	30	0.1891	31	
		Total	169			
		Allele Frequency	A=0.5651			
			G=0.4349	MAF		
		X (failed)	9 (5.1%)			
RRM1	rs9937	AA	33	0.2088	36	0.36
		AG	93	0.4963	86	
		GG	48	0.2950	51	
		Total	174			
		Allele Frequency	A=0.4569	MAF		
			G=0.5431			
		X (failed)	4 (2.2%)			
DCK	rs12648166	AA	27	0.1354	24	0.34
		AG	77	0.4651	82	
		GG	74	0.3995	71	
		Total	178			
		Allele Frequency	A=0.368	MAF		
			G=0.632			
		X (failed)	0			
Hcnt3	rs7853758	AA	3	0.0222	3	0.77
		AG	47	0.2534	45	
		GG	128	0.7244	128	
		Total	178			
		Allele Frequency	A=0.1489	MAF		
			G=0.8511			
		X (failed)	0			

Genes and SNPs by Sequenom Assay (continued)						
Marker	**RS #**	**Genotype**	**Observed Genotype n**	**Expected Genotype Frequency**	**Expected Genotype n***	**HWE p-value**
IGF1R	rs12437963	AA	123	0.6774	120	0.20
		GA	47	0.2913	51	
		GG	8	0.0313	5	
		Total	178			
		Allele Frequency	A=0.823			
			G=0.177	MAF		
		X (failed)	0			
MSH6	rs1800932	AA	119	0.6682	118	1.00
		GA	53	0.2985	53	
		GG	6	0.0333	5	
		Total	178			
		Allele Frequency	A=0.8174			
			G=0.1826	MAF		
		X (failed)	0			
IRS1	rs1801278	AA	4	0.0078	1	0.031
		GA	23	0.1606	28	
		GG	149	0.8316	146	
		Total	176			
		Allele Frequency	A=0.0881	MAF		
			G=0.9119			
		X (failed)	2 (1.1%)			
MRP2(ABCC5)	rs2273697	AA	10	0.0557	9	1.00
		GA	64	0.3606	64	
		GG	104	0.5838	103	
		Total	178			
		Allele Frequency	A=0.236	MAF		
			G=0.764			
		X (failed)	0			
DYPD	rs3918290	AA	0	0.000	0	1.00
		GA	1	0.006	0	
		GG	165	0.994	165	
		Total	166			
		Allele Frequency	A=0.003	MAF		
			G=0.997			
		X (failed)	12 (6.7%)			
MSH3	rs40139	AA	56	0.3488	55	1.00
		GA	77	0.4836	77	
		GG	27	0.1676	26	
		Total	160			
		Allele Frequency	A=0.5906			
			G=0.4094	MAF		
		X (failed)	18 (10.1%)			

Genes and SNPs by Sequenom Assay (continued)						
Marker	**RS #**	**Genotype**	**Observed Genotype n**	**Expected Genotype Frequency**	**Expected Genotype n***	**HWE p-value**
EXO1	rs4149909	AA	167	0.9495	167	1.00
		GA	9	0.0498	8	
		GG	0	0.0007	0	
		Total	176			
		Allele Frequency	A=0.9744			
			G=0.0256	MAF		
		X (failed)	2 (1.1%)			
MAPRE2	rs6507115	AA	3	0.0071	1	0.11
		GA	24	0.1543	27	
		GG	151	0.8386	149	
		Total	178			
		Allele Frequency	A=0.0843	MAF		
			G=0.9157			
		X (failed)	0			
Hent1	rs760370	AA	61	0.3486	61	0.88
		GA	87	0.4837	85	
		GG	29	0.1678	29	
		Total	177			
		Allele Frequency	A=0.5904			
			G=0.4096	MAF		
		X (failed)	1 (0.6%)			
TP73	rs9662633	AA	1	0.0018	0	0.26
		GA	13	0.0807	14	
		GG	164	0.9175	163	
		Total	178			
		Allele Frequency	A=0.0421	MAF		
			G=0.9579			
		X (failed)	0			
MGMT	rs9971190	AA	19	0.1267	22	0.33
		GA	88	0.4585	81	
		GG	70	0.4148	73	
		Total	177			
		Allele Frequency	A=0.3559	MAF		
			G=0.6441			
		X (failed)	1 (0.6%)			
KCNQ3	rs1457784	AA	8	0.0358	6	0.46
		CA	51	0.3069	54	
		CC	118	0.6573	116	
		Total	177			
		Allele Frequency	A=0.1893	MAF		
			C=0.8107			
		X (failed)	1 (0.6%)			

Genes and SNPs by Sequenom Assay (continued)						
Marker	**RS #**	**Genotype**	**Observed Genotype n**	**Expected Genotype Frequency**	**Expected Genotype n***	**Exact Test for HWE p-value**
MTHFR	rs1801131	AA	83	0.4635	82	0.73
		CA	75	0.4346	76	
		CC	19	0.1019	18	
		Total	177			
		Allele Frequency	A=0.6808			
			C=0.3192	MAF		
		X (failed)	1 (0.6%)			
CDA	rs2072671	AA	68	0.3924	69	0.63
		CA	87	0.4680	83	
		CC	23	0.1396	24	
		Total	178			
		Allele Frequency	A=0.6264			
			C=0.3736	MAF		
		X (failed)	0			
CDA	rs60369023	GG	177	1	177	
		Total	177			
		Allele Frequency	G=1			
		X (failed)	1			
IGF2	rs74050127	GG	178	1	178	
		Total	178			
		Allele Frequency	G=1			
		X (failed)	0			

Abbreviations: MAF, minor allele frequency; HWE, Hardy Weinberg Equilibrium
*Note: The sum of the expected genotypes might not equal the total sample size due to rounding.

Genotype method = SNaPshot Assay

Genes and SNPs by SNaPshot Assay

Marker	RS #	Genotype	Observed Genotype n	Expected Genotype Frequency	Expected Genotype n*	HWE p-value
TP73	rs2273953	CC	103	0.6334	107	0.093
		CT	63	0.3249	54	
		TT	3	0.0417	7	
		Total	169			
		Allele Frequency	C=0.7959			
			T=0.2041	MAF		
		X (failed)	9 (5.1%)			
PMS2L3	rs794378	CC	0	0.1572	26	<0.0001
		CT	134	0.4786	80	
		TT	35	0.3643	61	
		Total	169			
		Allele Frequency	C=0.3964	MAF		
			T=0.6036			
		X (failed)	9 (5.1%)			
hENT1	rs9394992	CC	82	0.5201	85	0.18
		CT	74	0.4021	66	
		TT	9	0.0777	12	
		Total	165			
		Allele Frequency	C=0.7212			
			T=0.2788	MAF		
		X (failed)	13 (7.3%)			
PLCG2	rs4889426	CC	74	0.4314	72	0.73
		CT	74	0.4508	76	
		TT	21	0.1178	19	
		Total	169			
		Allele Frequency	C=0.6568			
			T=0.3432	MAF		
		X (failed)	9 (5.1%)			
PMS2	rs17420802	AA	9	0.2775	46	<0.0001
		GA	159	0.4986	83	
		GG	0	0.2239	37	
		Total	168			
		Allele Frequency	A=0.5268			
			G=0.4732	MAF		
		X (failed)	10 (5.6%)			

Abbreviations: MAF, minor allele frequency; HWE, Hardy Weinberg Equilibrium

*Note: The sum of the expected genotypes might not equal the total sample size due to rounding.

Genotype method = Sanger Assay

Genes and SNPs by Sanger Assay

Marker	RS #	Genotype	Observed Genotype n	Expected Genotype Frequency	Expected Genotype n*	HWE p-value
MSH3	rs394592	CC	103	0.5890	101	0.52
		CT	58	0.3569	61	
		TT	11	0.0541	9	
		Total	172			
		Allele Frequency	C=0.7674			
			T=0.2326	MAF		
		X (failed)	6 (3.4%)			
DCK	rs4643786	CC	2	0.0021	0	0.04
		CT	12	0.0872	15	
		TT	161	0.9107	159	
		Total	175			
		Allele Frequency	C=0.0457	MAF		
			T=0.9543			
		X (failed)	3 (1.7%)			
KRAS	rs61764370	GG	3	0.0110	1	0.40
		TG	30	0.1874	32	
		TT	139	0.8016	137	
		Total	172			
		Allele Frequency	G=0.1047	MAF		
			T=0.8953			
		X (failed)	6 (3.4%)			
TYMS	rs34489327 (-/TTAAAG)	-/-	22	0.1086	19	0.31
		TTAAAG/-	72	0.4419	77	
		TTAAAG/TTAAAG	82	0.4495	79	
		Total	176			
		Allele Frequency	-/-=0.3295			
			TTAAAG =0.6705			
		X (failed)	2 (1.1%)			

Abbreviations: MAF, minor allele frequency; HWE, Hardy Weinberg Equilibrium

*Note: The sum of the expected genotypes might not equal the total sample size due to rounding.

Aim 4: TRP 169: Ribonucleotide reductase in cervix cancers: This project restricts its data to two cervical cancer trials: RTOG 0116 and 0128. The aim is to associate ribonucleotide reductase (RNR) M2 and p53R2 expression with survival.

Evaluation of Ribonucleotide Reductase Expression in Cervix Cancer

Cervical cancer, which is commonly positive for human papillomavirus (HPV) and abnormal p53 signaling, is an aggressive malignancy marked by higher rates of incomplete radiochemotherapeutic response and poorer disease-specific survival if ribonucleotide reductase (RNR) is overactive. As an important supplier of deoxyribonucleotide diphosphates (dNDPs) used as building blocks for DNA, RNR has emerged as a therapeutic target in cervical cancer. RNR in its M1-M2 form supplies dNDPs for S-phase-specific DNA replication. RNR in its M1-M2b form supports cell-cycle independent dNDP demands and DNA damage responses. Regulation of RNR catalytic activity in resting cells and in cycling cells is limited by differential expression and degradation of M1, M2, and M2b. One unanswered question is how RNR subunits influence radiochemotherapeutic outcome in women with cervical cancer. One possible answer might be that the subunits facilitate fixing of damaged DNA and suppress the death-provoking effects of radiochemotherapy. This retrospective analysis was designed to evaluate whether pretherapy RNR M1, M2, and M2b immunohistochemical overexpression was associated with shortened disease-free and overall survival in two clinical trials conducted by the Radiation Therapy Oncology Group (RTOG).

Immunohistochemistry for RNR M1, M2, M2b (0-3+) was conducted on cervical cancer tissues retrieved from women treated on RTOG 0116 and 0128. Patients entered onto RTOG 0116 (node-positive stage IA-IVA) received weekly cisplatin (40 mg/m^2) and extended-field radiation then brachytherapy (85 Gy). RTOG 0128 patients (node-positive or bulky $\geq$ 5 cm stage IB-IIA, or stage IIB-IVA) received cisplatin (75 mg/m^2) and 5-FU (4-day 1 gm/m^2) on days 1, 23, & 43 during daily pelvic radiation then brachytherapy (85 Gy), plus celecoxib (400 mg twice daily, day 1 through 1 year). Disease-free survival (DFS) was any failure and all-cause death as measured from enrollment date to first failure, death, or last censored follow-up. DFS was estimated univariately with the Kaplan-Meier method and Cox proportional hazards models was used to assess the impact of RNR expression on DFS. All analyses were completed using SAS (version 9.2 for Windows, SAS Institute, Cary, NC) and R (version 2.14 for windows, R Foundation for Statistical Computing).

RTOG 0116 accrued 45 patients between August, 2001, and March, 2007. Of these 45 patients, 13 were eligible and had suitable cervical cancer tissue banked for immunoreactivity studies. RTOG 0128 enrolled 84 patients between August, 2001 and March 2004. Of these 84 patients, 38 patients were eligible and had sufficient tissue banked for immunohistochemical analysis. Fifty-one patients, therefore, were included in this analysis. All analyzed cervical cancer tissue was obtained prior to radiochemotherapy. Patient demographic and efficacy were not statistically significantly different between the patients with and without suitable cervical cancer tissue. Patient demographic and tumor variables were not statistically significantly different between the

RNR M1 (0-1+ v. 2-3+), M2 (0-2+ v. 3+), and M2b (0-1+ v. 2-3+) staining intensity subgroups. Median follow-up for all patients is 24 months (minimum-maximum, 2 to 44 months). Thirteen (25%) local and/or regional disease relapses have been reported. Fifteen deaths have been recorded.

The pretherapy expression of RNR M1 in the cytosol was low (0-1+) in the majority of cervical cancers (44 [86%] of 51). RNR M1 overexpression (2-3+) was not associated with an increased incidence of pelvic ($p = 0.17$) or para-aortic ($p = 0.33$) lymph node metastases at cervical cancer diagnosis. There were no deaths, and two (29%) disease-related events in seven patients with RNR M1 overexpression. In this dataset, high (2-3+) levels of RNR M1 were not associated with shorter DFS (log-rank $p = 0.38$) or OS (log-rank $p = 0.11$). RNR M1 expression was not associated with DFS in any of the two-variable Cox models. A high 3+ level of RNR M2 expression seen in the cytosol occurred in most (41 [80%] of 51) cervical cancers. Pelvic ($p = 0.43$) or para-aortic ($p = 0.35$) lymph node metastases at diagnosis were not statistically significantly more common when RNR M2 expression was high (3+). Of those patients that had relapse or death, more patients had RNR M2 overexpression (3+) (16/22=73%) than had RNR M2 (0/1/2+) immunoreactivity (6/22=27%). Of those patients that died, more patients had RNR M2 overexpression (3+) (10/15=67%) than had RNR M2 (0/1/2+) immunoreactivity (5/15=33%). Despite these findings, RNR M2 overexpression (3+) was not statistically associated with lower estimate of DFS ($p = 0.19$) or OS ($p = 0.07$). After adjusting for M2 status, pelvic node-positive women were 4.7 (95% CI: 1.9-11.4) times more likely to relapse or die ($p = 0.0006$). The high (2-3+) level of RNR M2b in the cytosol was seen in more than half (30 [59%] of 51) of cervical cancers. Pelvic ($p = 0.26$) or para-aortic ($p = 0.28$) lymph nodes were not more common when a high (2-3+) level of RNR M2b was detected. Of the 22 patients with relapses or deaths, high (2-3+) levels of RNR M2b were more common ($n = 14$, 64%). This did not translate into a statistically significantly shortened DFS in univariate ($p = 0.69$) analysis. OS was also not associated with RNR M2b overexpression in univariate ($p = 0.16$) analysis. After adjusting for M2b status, pelvic node-positive women had an increased hazard for relapse or death (HR: 4.9, 95% CI: 2.0-12.0; $p=0.0005$).

This analysis provides a much needed proof-of-concept that pretherapy RNR M1, M2, and M2b expression sharpens the thinking about radiochemotherapy response in women with cervical cancer. On the basis of these results, prospective collections of uterine cervix tissue adequately powered to study RNR immunoreactivity, DFS, and OS are being considered.

Aim 5: TRP 91: Expression of receptors in bladder cancers: The final project utilizes multiple RTOG bladder sparing trials, particularly muscle-invasive bladder cancers treated with selective bladder preservation. The objective is to correlate the level of expression with the primary tumor site by immunohistochemical staining of VEGF and VEGF receptors, Flt-1 and Flk-1, with response, recurrence and survival.

Most patients with localized muscle-invasive bladder cancer are treated with radical

cystectomy in the US. Bladder preservation trials have been designed and conducted by the RTOG for patients with muscle invasive bladder cancer who were otherwise candidates for radical cystectomy. The overall survival for patients treated with bladder preserving chemo and radiation therapy at 5 years ranges from 45% to 52% and of the surviving patients 54% to 67% have a tumor-free normally functioning bladder. While bladder preservation therapy can lead to improved quality of life, up to one third of these patients will require cystectomy for persistent or recurrent disease. This analysis sought to discover biomarkers that might predict patients who will have total tumor eradication in their pelvis by combining maximal TURBT and chemoradiation. To this end, the vascular endothelial growth factor (VEGF) family of proteins which are known to be important determinants of angiogenesis were investigated. VEGF promotes endothelial mitogenesis and migration, extracellular matrix remodeling, increased vascular permeability, and maintenance of newly formed vasculature. While VEGF-A is important for the formation of blood vessels, such as during development or in pathological conditions, VEGF-B plays a role in the maintenance of newly formed blood vessels during pathological conditions. VEGF-B mRNA has not yet been shown to be expressed either in normal urothelium or in bladder cancer. VEGF-C and –D both appear to be active in angiogenesis, lymphangiogenesis and endothelial cell growth and VEGF-C may affect the permeability of blood vessels. Patients with T2 or lower stage, low level of cytoplasmic VEGF-C and absence of simultaneous multifocal bladder Tis have been associated with high overall survival and disease-specific survival rate. Both VEGF-C and –D have been shown to regulate lymphangiogenesis and angiogenesis and influence metastasis-free survival of bladder cancer patients. VEGF ligands lead to receptor dimerization and subsequent signal transduction by binding to their associated receptors. Given the importance of VEGF ligands and receptors in bladder cancer biology, it was hypothesized that VEGF biomarkers might be associated with response to induction chemoradiation in bladder preservation therapy patients. To test this hypothesis, immunofluoresence staining of VEGF ligands A, B, C, D and the VEGF receptors R1 and R2 was performed on TURBT formalin fixed paraffin embedded (FFPE) tissue samples collected before initiation of chemoradiation therapy.

RTOG completed 4 protocols (RTOG 8802, 8903, 9506, & 9706) from 1988 to 1999 treating clinical T2-4a MIBC patients with selective bladder preservation using transurethral surgery (TURBT) plus cisplatin-containing induction & consolidation chemoradiation regimens with tumor response evaluation, reserving radical cystectomy for invasive tumor persistence or recurrence. Molecular markers on the VEGF angiogenesis pathway were evaluated for association with efficacy outcomes. HistoRx AQUA® platform and fluorescence immunohistochemistry were used to quantify the expression of VEGF-R1,-R2, and VEGF-A,-B,-C, and -D in the tumor, cytoplasmic and nuclear compartments of each TMA core. AQUA scores were available for VEGF–A, VEGF-B, VEGF-C, VEGF-D, VGEF-R1, and VEGF-R2 in both the nucleus and cytoplasm. Each VEGF expresser value was analyzed as a categorical variable using its median as the cut point ($<$ median vs. $\geq$ Median) and outcomes of interest were complete response (CR), local failure (LF), distant failure (DF) and overall survival (OS). The association between VEGF (nucleus/cytoplasm) expression level and complete response was evaluated using logistic regression. For OS the Kaplan-Meier method was used to

estimate the rates, with the log-rank test used to compare expresser groups (high versus low). The Cox proportional hazards (PH) model was used to estimate the hazard ratio (HR) associated with each endpoint. LF was defined as persistent local disease, or local or regional relapse of the disease, and DF was defined as distant metastases; estimates for both were calculated using Gray's method by taking into consideration of possible competing risks. All analyses were completed using SAS (version 9.2 for Windows, SAS Institute, Cary, NC) and R (version 2.14 for windows, R Foundation for Statistical Computing).

A total of 306 bladder patients registered to the four trials, of which 294 were clinically eligible. VEGF marker data was available for approximately 15% of patients: The number (percentage) of patients on whom each marker was measured was: VEGF-A [34(11.6%)], VEGF-B [35(11.9%)], VEGF-C [33(11.2%)], VEGF-D [37(12.6%)], VEGF-R1 [38(12.9%)], and VEGF-R2 [37(12.6%)]. The median follow-up times (years) for all patients were: VEGF-A [3.1], VEGF-B [3.2], VEGF-C [3.2], VEGF-D [3.1], VEGF-R1 [3.1], and VEGF-R2 [3.2]. There are no statistically significant differences in the baseline characteristics of the patients with and without biomarker data. The CR, LF and DF rates between patients with missing and determined VEGF data were similar. The OS estimates for patients with missing and determined VEGF-A, VEGF-C, VEGF-D and VEGF-R2 were similar while they slightly differed for VEGF-B [3 years, 63.5% vs. 58.6%, p=0.04] and VEGF-R1 [3 years, 63.9% vs. 56.5%, p=0.04]. There was no difference in the complete response rate with different staining levels of the VEGF ligands or receptors. Higher levels of cytoplasmic VEGF-B, VEGF-C, and VEGF-R2 were statistically significantly associated with decreased overall survival rates. Three-year OS rates (95% CI) were 43.7% (20.5%-64.8%) for patients with high VEGF-B expression compared to 75% (46.3%-89.8%)for those with low VEGF-B expression (p=0.01) . Patients with high VEGF-B cytoplasm expression have a significant increase in the risk of death compared to those patients with low VEGF-B expression (HR =2.83, 95% CI [1.22, 6.59], p-value=0.02). Patients with low VEGF-C cytoplasm expression have a 3-year OS estimate of 86.7% (17.3%-62.2%) vs. 40.2% (56.4%-96.5%) for those with higher expression levels (p=0.01). Those with high VEGF-C cytoplasm expression have significant increased risk of death opposite to patients with low VEGF-C cytoplasm expression (HR =3.10, 95% CI [1.31, 7.36], p-value=0.02). The patients with low VEGF-R2 cytoplasm expression levels have a 3-year OS rate of 66.7% (40.4%-83.4%) , while patients with high expression levels have a 3-year OS of 49.7% (25.4%-70.0%; p =0.02). Patients with high VEGF-R2 cytoplasm expression were associated with significant increased risk of death compared to those with low expression levels (HR =2.47, 95% CI [1.10, 5.55], p-value=0.03). Higher levels of VEGF-B cytoplasm are associated with higher rates of DF (Gray's p-value=0.02). The 3-year DF estimates for high VEGF-B cytoplasm expression were 39.5% (15.3%- 63.7%) compared to 12.5% (0.0%-29.4%) for patients with low VEGF-B cytoplasm expression. Patients with high VEGF-B cytoplasm expression have a significantly increased DF risk compared to those with low levels (HR=4.23, 95% CI:[1.22-14.62], p-value=0.02). There was no association of VEGF-C cytoplasm expression and DF (p=0.86). The 3-year DF estimates for high VEGF-C cytoplasm expression were 29.0% (6.7%, 51.2%) compared to 20.0% (0.0%, 41.1%) for patients with low VEGF-C cytoplasm expression. Of note, there is an early

observed DF effect in between the VEGF-C expression levels. The association between cytoplasmic VEGF-D expression and DF rates did not reach statistical significance (p=0.14). VEGF-A and VEGF-R1 levels were not associated with DF or OS. There was no significant association between expression of any of the tested VEGF ligands or receptors LF.

In summary these results show VEGF-B, C, and R2 to be biomarkers for overall survival and VEGF-B a marker for distant metastasis. Those results are consistent with published VEGF functions. While VEGF biomarkers did not predict for response to induction chemoradiation for bladder preservation patients the association of VEGF with outcome suggests that a) VEGF could be of functional relevance for bladder cancer tumorigenesis, tumor maintenance, and resistance to therapy and that b) the addition of anti-angiogenic therapies should be evaluated for selected bladder preservation patients with VEGF overexpression and that c) lower expression of VEGF biomarkers selects for a patient population with improved outcome after bladder preservation therapy and therefore VEGF expression should be considered for further evaluation as a biomarker to determine which patients are the best candidates for bladder preservation therapy.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Ribonucleotide Reductase Expression in Cervix Cancer: a Radiation Therapy Oncology Group Translational Science Analysis	Charles A. Kunos, Kathryn Winter, Adam P. Dicker, William Small Jr., Fadi W. Abdul-Karim, Dawn Dawson, Anuja Jhingran, Richard Valicenti, Joanne B. Weidhaas and David K. Gaffney	International Journal of Gynecologic Oncology	November 2012	☐Submitted ☐Accepted ■Published
2. Bladder Preservation Therapy	Tim Lautenschlaeger, Asha George,	The Oncologist	December 2012	☐Submitted ☐Accepted

for Muscle-Invading Bladder Cancers on Radiation Therapy Oncology Group Trials 8802, 8903, 9506, and 9706: Vascular Endothelial Growth Factor B Overexpression Predicts for Increased Distant Metastasis and Shorter Survival	Alexander C. Klimowicz, Jason A. Efstathiou, Chin-Lee Wu, Howard Sandler, William Shipley, William J. Tester, Michael P. Hagan, Anthony M. Magliocco, Arnab Chakravarti,			■Published
3. Glycogen Synthase Kinase 3 beta (GSK3β) predicts survival in adenocarcinoma of the pancreas	Edgar Ben-Josef, Asha George, William F. Regine, , Ross Abrams, Meredith Morgan, Dafydd Thomas, Paul L. Schaefer, Thomas A. DiPetrillo, Mitchel Fromm, William Small, Jr., Samir Narayan, Kathryn Winter, Chandan Guha, Terence M. Williams,	Journal of Clinical Oncology	November 2014	■Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

There will be a manuscript submitted for the *Evaluation of p16 and p53 Expression on Clinical Outcome in Patients with Anal Cancer Treated with Chemoradiotherapy: An Analysis of RTOG 98-11* analysis.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.

Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The results from these analyses identified biomarkers in cervix cancer, bladder cancer,

pancreas cancer and anal cancer that were associated with efficacy outcomes. Some of the results, such as the cervix RNR M1, M2, and M2b expressions and the bladder VEGF expressions have added to the literature and will provide for hypotheses in future translational projects. The results from the phase III pancreas and anal canal trials may provide a basis for stratification in future clinical trials in those disease sites.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME Winter, Kathryn	POSITION TITLE Director of Statistics
eRA COMMONS USER NAME (credential, e.g., agency login) NONE	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Shippensburg University, Shippensburg, PA	B.A.	1993	Mathematics
University of Florida, Gainesville, FL	M.S.	1995	Statistics

A. Positions and Honors

Positions and Employment

1993-1995 Teaching Assistant, Department of Statistics, University of Florida, Gainesville, FL
1995-1999 Statistician, RTOG Statistical Center, American College of Radiology, Philadelphia, PA
1999-2006 Senior Statistician, RTOG Statistical Center, American College of Radiology, Philadelphia, PA
2001-2002 Adj. Professor, Department of Mathematics and Computer Science, St. Joseph's University, Philadelphia, PA
2002-2006 Adjunct Professor, College of Graduate Studies, Thomas Jefferson University, Philadelphia, PA
2005-present Statistical Representative, NCI GI Pancreas Task Force
2006-2007 Acting Senior Director, Research & Operations, RTOG Statistical Center, American College of Radiology (ACR), Philadelphia, PA
2006-2007 Acting Group Statistician, RTOG Statistical Center, ACR, Philadelphia, PA
2006-present Statistical Representative, NCI GYN Cancer Steering Committee
2006-present RTOG Representative, NCI Group [Biospecimen] Banking Committee
2007-2010 Statistical Representative, NCI CIRB
2007-present Statistical Representative, NCI GYN Cervical and Uterine Task Forces
2007-present Statistical Representative, NCI GI Pancreas Task Forces
2008-2009 Acting Group Statistician, RTOG Statistical Center, ACR, Philadelphia, PA
2007-2014 Director, RTOG Statistical Center, ACR, Philadelphia, PA
2014-present Director, NRG Oncology Statistics and Data Management Center, ACR, Philadelphia, PA

B. Selected Peer-reviewed Publications (from a total of 80)

1. Lawton, CA, **Winter K**, et al: Androgen Suppression Plus Radiation vs. Radiation Alone for Patients with D1 (pN+) Adenocarcinoma of the Prostate (Results Based on a National

Prospective Randomized Trial RTOG 85-31). *Int J Radiat Oncol Biol Phys*, 38(5):931-939, 1997.
2. Horwitz E, **Winter K**, et al: Subset Analysis of RTOG 85-31 and 86-10 Indicates an Advantage for Long-Term Versus Short-Term Adjuvant Hormones for Patients with Locally Advanced Non-Metastatic Prostate Cancer Treated with Radiation Therapy. Int J Radiat Onco Biol Phys 49: 947-956, 2001.
3. Pilepich M, **Winter K**, et al: Phase III Radiation Therapy Oncology Group (RTOG) Trial 86-10 of Androgen Deprivation Adjuvant to Definitive Radiotherapy in Locally Advanced Carcinoma of the Prostate. Int J Radiat Onco Biol Phys 50(5): 1243-1252, 2001.
4. Eifel P, **Winter K,** et al: Pelvic Radiation with Concurrent Chemotherapy versus Pelvic and Para-Aortic Radiation for High-Risk Cervical Cancer: An Update of RTOG 90-01. Int J Radiat Onco Biol Phys. 22(5): 872-880, 2004.
5. Gaffney D, **Winter K,** et al: Feasibility of RNA Collection for Micro-Array Gene Expression Analysis in the Treatment of Cervical Carcinoma: A Scientific Correlate of RTOG C-0128. Gynecologic Oncology 97(2): 607-611, 2005.
6. Regine W, **Winter, K**, et al: Fluorouracil vs Gemcitabine Chemotherapy Before and After Fluorouracil-Based Chemotherapy Following Resection of Pancreatic Adenocarcinoma: A Randomized Controlled Trial. *JAMA*, **299,** 1019-1026, 2008.
7. Ajani J, **Winter, K**, et al: (Fluorouracil, Mitomycin, and Radiotherapy vs Fluorouracil, Cisplatin, and Radiotherapy for Carcinoma of the Anal Canal: A Randomized Controlled Trial. *JAMA*, 299, 1914-1921, 2008.
8. Weidhaas, J., Li, S., **Winter, K.**, et al: Changes in Gene Expression Predicting Local Control in Cervical Cancer: Results from RTOG 0128. *Clin Cancer Res*, 15, 4199-4206, 2009.
9. Wong, S., **Winter, K.**, et al.: RTOG 0247: A Randomized Phase II Study of Neoadjuvant Capecitabine and Irinotecan or Capecitabine and Oxaliplatin with Concurrent Radiation Therapy for Locally Advanced Rectal Cancer. *Int J Radiat Oncol Biol Phys*, 82 (4):1367-1375, 2012.
10. Doll CM, **Winter K,** et al. COX-2 expression and survival in patients with locally advanced cervical cancer treated with chemoradiotherapy and celecoxib: a quantitative immunohistochemical analysis of RTOG C0128. *Int J Gynecol Cancer*. 2013 Jan;23(1):176-83.
11. Kunos CA, **Winter K,** et al. Ribonucleotide reductase expression in cervical cancer: a radiation therapy oncology group translational science analysis. *Int J Gynecol Cancer*. 2013 May;23(4):615-21.
12. McAllister F, Pineda DM, Jimbo M, Lal S, Burkhart RA, Moughan J, **Winter KA,** et al. dCK expression correlates with 5-fluorouracil efficacy and HuR cytoplasmic expression in pancreatic cancer: a dual-institutional follow-up with the RTOG 9704 trial. *Cancer Biol Ther*. 2014 Jun 1;15(6):688-98.
13. Lee LJ, Ratner E, Uduman M, **Winter K,** et al. The KRAS-variant and miRNA expression in RTOG endometrial cancer clinical trials 9708 and 9905. *PLoS One*. 2014 Apr 14;9(4):e94167.
14. Siegel EM, Eschrich S, **Winter K,** et al. Epigenomic characterization of locally advanced anal cancer: a radiation therapy oncology group 98-11 specimen study. Dis Colon Rectum. 2014 Aug;57(8):941-57.